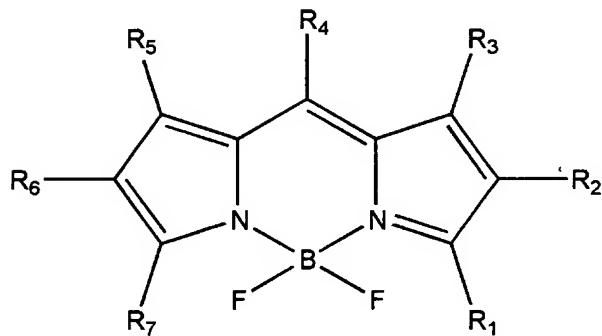


What is claimed is

1. A method of labeling poly(amino acids) comprising the steps of:
  - 5    a. separating poly(amino acids) by gel electrophoresis, resulting in separated poly (amino acids);
    - b. transferring said separated poly(amino acids) to a solid support, resulting in immobilized poly(amino acids) ;
    - 10    c. combining said immobilized poly(amino acids) on said solid support with a labeling mixture that comprises one or more chemically reactive dipyrrometheneboron difluoride dyes of the formula:



wherein each of R<sup>1</sup> through R<sup>7</sup> are independently selected from the group consisting of H, halogen, L-Rx, and substituted or unsubstituted C<sub>1</sub>-C<sub>6</sub> alkyl [carboxylic acid, sulfonic acid, or halogen], aryl, arylethenyl, arylbutadienyl, and heteroaryl [C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl];

provided that one or more of R<sup>1</sup> through R<sup>7</sup> is H,

two or more of R<sup>1</sup> through R<sup>7</sup> is nonhydrogen, and

only one of R<sup>1</sup> through R<sup>7</sup> is -L-Rx, where L is a spacer having 1-24 nonhydrogen atoms selected from the group consisting of C, N, O, P, and S and is composed of any combination of single, double, triple or aromatic carbon-carbon bonds, carbon-

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nitrogen bonds, nitrogen-nitrogen bonds, carbon-oxygen bonds, carbon-sulfur bonds, phosphorus-oxygen bonds, and phosphorus-nitrogen bonds; and Rx is a reactive group that is a maleimide or a succinimidyl ester of a carboxylic acid; such that the dipyrrometheneboron difluoride dye has an absorption maximum, between 495 nm and 640 nm;

- d. incubating the immobilized poly(amino acids) in the labeling mixture for a sufficient time for the dyes to form a covalent bond with said poly(amino acids), resulting in labeled poly(amino acids).

10

2. A method, as claimed in Claim 1, wherein for the dipyrrometheneboron difluoride dye, Rx is a succinimidyl ester of a carboxylic acid.

15

3. A method, as claimed in Claim 1, wherein said solid support is made of solvent-resistant materials that are selected from the group consisting of nylon, poly(vinylidene difluoride), glass, plastics, and their derivatives.

20

4. A method, as claimed in Claim 3, wherein said solid support is made of materials that are poly(vinylidene difluoride).

25

5. A method, as claimed in Claim 1, wherein said poly(amino acids) immobilized on said solid support has a molecular weight of 500 to 200,000 Daltons.

6. A method, as claimed in Claim 1, wherein said dye is present in the labeling mixture at a concentration of 0.10 micromolar to 10 micromolar.

30

7. A method, as claimed in Claim 1, wherein for the dipyrrometheneboron difluoride dye, R<sup>1</sup> is methyl or -L-Rx; R<sup>2</sup> is H, bromine, or -L-Rx; R<sup>3</sup> is H or methyl; R<sup>4</sup> is H or -L-Rx; R<sup>5</sup> is H, methyl, or phenyl; R<sup>6</sup> is H or bromine; and R<sup>7</sup> is methyl, phenyl, alkoxyphenyl, phenylethenyl, phenylbutadienyl pyrrolyl, or thiényl; where -L- is -(CH<sub>2</sub>)<sub>2</sub>- , -(CH<sub>2</sub>)<sub>4</sub>- , -OCH<sub>2</sub>C(O)NH(CH<sub>2</sub>)<sub>5</sub>- , -(CH<sub>2</sub>)<sub>2</sub>-C(O)NH(CH<sub>2</sub>)<sub>5</sub>- ,

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$-(CH_2)_2C_6H_4OCH_2C(O)NH(CH_2)_5$ ;  
and Rx is a succinimidyl ester of a carboxylic acid.

8. A method, as claimed in Claim 7, further comprising adding a specific binding pair

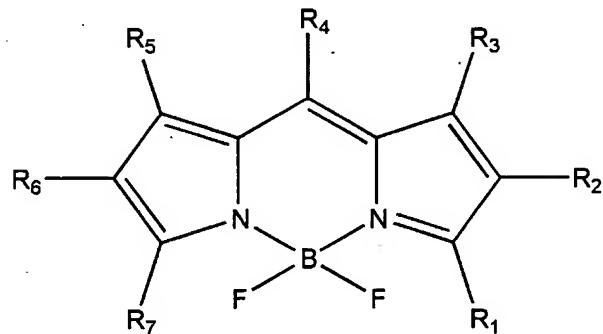
5 member that contains a label and that binds selectively to a target within the immobilized poly(amino acids) that is its complementary binding pair.

✓ 9. A method of labeling poly(amino acids) bound to aptamers comprising the steps of:

a. incubating immobilized aptamers with poly(amino acids) for a sufficient time to allow  
10 said poly(amino acids) to bind to their specific aptamers, resulting in immobilized poly(amino acids);

b. removing unbound poly(amino acids) that are not immobilized,

15 c. combining said immobilized poly(amino acids) with a labeling mixture that comprises one or more chemically reactive dipyrrometheneboron difluoride dyes of the formula:



wherein each of R<sup>1</sup> through R<sup>7</sup> are independently selected from the group consisting  
20 of H, halogen, L-Rx, and substituted or unsubstituted C<sub>1</sub>-C<sub>6</sub> alkyl, aryl, arylethenyl,  
arylbutenyl, and heteroaryl;

provided that one or more of R<sup>1</sup> through R<sup>7</sup> is H,

two or more of R<sup>1</sup> through R<sup>7</sup> is nonhydrogen, and

only one of R<sup>1</sup> through R<sup>7</sup> is -L-Rx, where L is a spacer having 1-24 nonhydrogen  
25 atoms selected from the group consisting of C, N, O, P, and S and is composed of  
any combination of single, double, triple or aromatic carbon-carbon bonds, carbon-

nitrogen bonds, nitrogen-nitrogen bonds, carbon-oxygen bonds, carbon-sulfur bonds, phosphorus-oxygen bonds, and phosphorus-nitrogen bonds; and Rx is a reactive group that is a maleimide or a succinimidyl ester of a carboxylic acid; such that the dipyrrometheneboron difluoride dye has an absorption maximum between 495 nm and 640 nm;

- 5                   d. incubating the immobilized poly(amino acids) with the labeling mixture for a sufficient time to form a covalent bond between the dipyrrometheneboron difluoride dye and said immobilized poly(amino acids), resulting in labeled poly(amino acids) that are bound to the  
10                  aptamers.

15                 10. A method, as claimed in Claim 9, wherein the dipyrrometheneboron difluoride dye's chemically reactive group is a succinimidyl ester of a carboxylic acid.

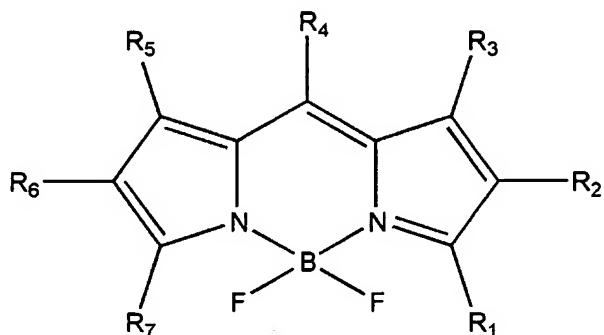
20                 11. A method, as claimed in Claim 9, wherein said dipyrrometheneboron difluoride dye is present in the combined labeling mixture at a concentration of 0.10 micromolar to 10 micromolar.

25                 12. A method, as claimed in Claim 9, wherein for the dipyrrometheneboron difluoride dye, R<sup>1</sup> is methyl or -L-Rx; R<sup>2</sup> is H, bromine, or -L-Rx; R<sup>3</sup> is H or methyl; R<sup>4</sup> is H or -L-Rx; R<sup>5</sup> is H, methyl, or phenyl; R<sup>6</sup> is H or bromine; and R<sup>7</sup> is methyl, phenyl, alkoxyphenyl, phenylethenyl, phenylbutadienyl pyrrolyl, or thienyl; where -L- is -(CH<sub>2</sub>)<sub>2</sub>-, -(CH<sub>2</sub>)<sub>4</sub>-, -OCH<sub>2</sub>C(O)NH(CH<sub>2</sub>)<sub>5</sub>-, -(CH<sub>2</sub>)<sub>2</sub>-C(O)NH(CH<sub>2</sub>)<sub>5</sub>-, -(CH)<sub>2</sub>C<sub>6</sub>H<sub>4</sub>OCH<sub>2</sub>C(O)NH(CH<sub>2</sub>)<sub>5</sub>-, and Rx is a succinimidyl ester of a carboxylic acid.

30                 13. A method, as claimed in Claim 12, further comprising adding a specific binding pair member that contains a label and that binds selectively to a target within the immobilized poly(amino acids) that is its complementary binding pair.

14 A method of labeling immobilized poly(amino acids) in an array comprising the steps  
of:

- 5       a. combining an array of immobilized poly(amino acids) with a labeling mixture that  
comprises one or more chemically reactive dipyrrometheneboron difluoride dyes of  
the formula



10       wherein each of R<sup>1</sup> through R<sup>7</sup> are independently selected from the group consisting  
of H, halogen, L-Rx, and substituted or unsubstituted C<sub>1</sub>-C<sub>6</sub> alkyl, aryl, arylethenyl,  
arylbutadienyl, and heteroaryl;

15       provided that one or more of R<sup>1</sup> through R<sup>7</sup> is H,

      two or more of R<sup>1</sup> through R<sup>7</sup> is nonhydrogen, and

      only one of R<sup>1</sup> through R<sup>7</sup> is -L-Rx, where L is a spacer having 1-24 nonhydrogen  
atoms selected from the group consisting of C, N, O, P, and S and is composed of  
any combination of single, double, triple or aromatic carbon–carbon bonds, carbon–  
nitrogen bonds, nitrogen–nitrogen bonds, carbon–oxygen bonds, carbon–sulfur  
bonds, phosphorus–oxygen bonds, and phosphorus–nitrogen bonds; and Rx is a  
reactive group that is a maleimide or a succinimidyl ester of a carboxylic acid;

20       such that the dipyrrometheneboron difluoride dye has an absorption maximum  
between 495 nm and 640 nm;

- 25       b. incubating said array with the labeling mixture for a sufficient time to form a covalent  
bond between the dipyrrometheneboron difluoride dye and said immobilized poly(amino  
acids), resulting in the array of poly(amino acids) being labeled.

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15. A method, as claimed in Claim 14, wherein for the dipyrrometheneboron difluoride dye, Rx is a succinimidyl ester of a carboxylic acid.

16. A method, as claimed in Claim 14, wherein said dipyrrometheneboron difluoride dye is present in the labeling mixture at a concentration of 0.10 micromolar - 10 micromolar.

5

17. A method, as claimed in Claim 14, wherein for the dipyrrometheneboron difluoride dyes, R<sup>1</sup> is methyl or -L-Rx; R<sup>2</sup> is H, bromine, or -L-Rx; R<sup>3</sup> is H or methyl; R<sup>4</sup> is H or -L-Rx; R<sup>5</sup> is H, methyl, or phenyl; R<sup>6</sup> is H or bromine; and R<sup>7</sup> is methyl, phenyl, alkoxyphenyl, phenylethenyl, phenylbutatdienyl pyrrolyl, or thienyl;

10 where -L- is -(CH<sub>2</sub>)<sub>2</sub>-, -(CH<sub>2</sub>)<sub>4</sub>-, -OCH<sub>2</sub>C(O)NH(CH<sub>2</sub>)<sub>5</sub>-, -(CH<sub>2</sub>)<sub>2</sub>-C(O)NH(CH<sub>2</sub>)<sub>5</sub>-,

(CH<sub>2</sub>)<sub>2</sub>C<sub>6</sub>H<sub>4</sub>OCH<sub>2</sub>C(O)NH(CH<sub>2</sub>)<sub>5</sub>;

and Rx is a succinimidyl ester of a carboxylic acid.

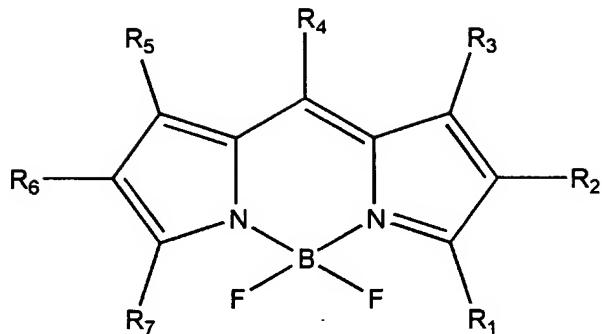
15 18. A method, as claimed in Claim 17, further comprising adding specific binding pair member that contains a label and that binds selectively to a target within the immobilized poly(amino acids) that is its complementary binding pair.

20 19. A method, as claimed in Claim 14, further comprising adding specific binding pair member that contains a label and that binds selectively to a target within the immobilized poly(amino acids) that is its complementary binding pair.

20 A method of detecting poly(amino acids) comprising the steps of:

a. combining poly(amino acids) immobilized on a solid support; with a labeling mixture

25 that comprises one or more chemically reactive dipyrrometheneboron difluoride dyes of the formula



wherein each of R<sup>1</sup> through R<sup>7</sup> are independently selected from the group consisting of H, halogen, L-Rx, and substituted or unsubstituted C<sub>1</sub>-C<sub>6</sub> alkyl, aryl, arylethenyl, 5 arylbutadienyl, and heteroaryl;

provided that one or more of R<sup>1</sup> through R<sup>7</sup> is H,

two or more of R<sup>1</sup> through R<sup>7</sup> is nonhydrogen, and

only one of R<sup>1</sup> through R<sup>7</sup> is -L-Rx, where L is a spacer having 1-24 nonhydrogen atoms selected from the group consisting of C, N, O, P, and S and is composed of any combination of single, double, triple or aromatic carbon-carbon bonds, carbon-nitrogen bonds, nitrogen-nitrogen bonds, carbon-oxygen bonds, carbon-sulfur bonds, phosphorus-oxygen bonds, and phosphorus-nitrogen bonds; and Rx is a reactive group that is a maleimide or a succinimidyl ester of a carboxylic acid; such that the dipyrrrometheneboron difluoride dye has an absorption maximum between 495 nm and 640 nm;

b. incubating said immobilized poly(amino acids) with the labeling mixture for a sufficient time to form a covalent bond between the dipyrrrometheneboron difluoride dye and said immobilized poly(amino acids) resulting in labeled poly(amino acids);

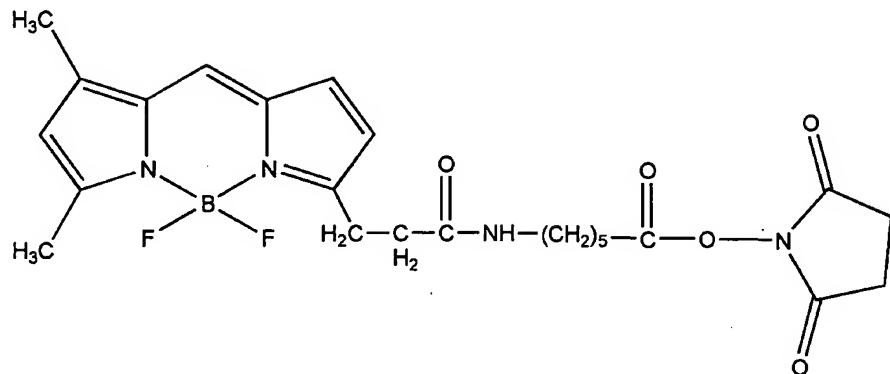
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c. removing unbound dipyrrrometheneboron difluoride dyes ;

d. illuminating said labeled poly(amino acids) to yield a fluorescent optical response to detect the corresponding labeled poly(amino acids).

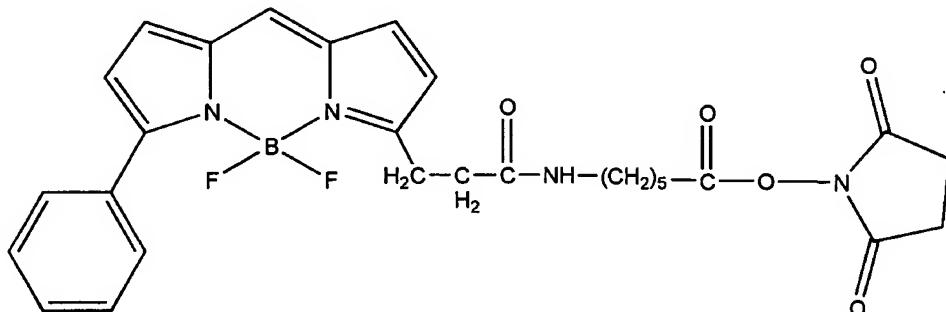
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21. A method, as claimed in Claim 20, wherein the dipyrrometheneboron difluoride dye has the formula:



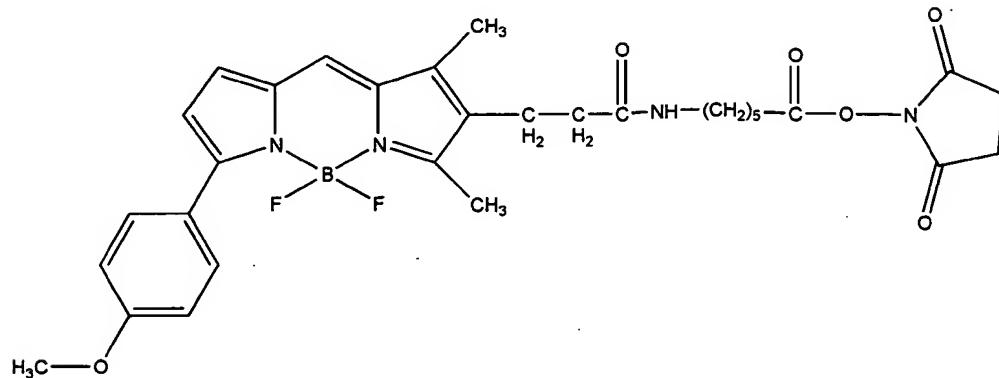
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22. A method, as claimed in Claim 20, wherein the dipyrrometheneboron difluoride dye has the formula:

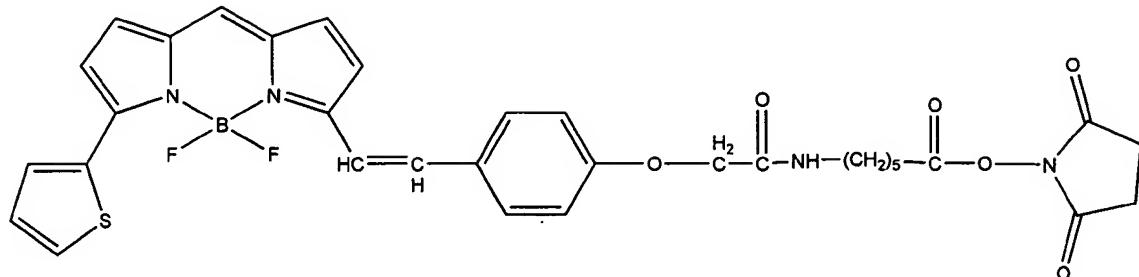


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23. A method, as claimed in Claim 20, wherein the dipyrrometheneboron difluoride dye has the formula:

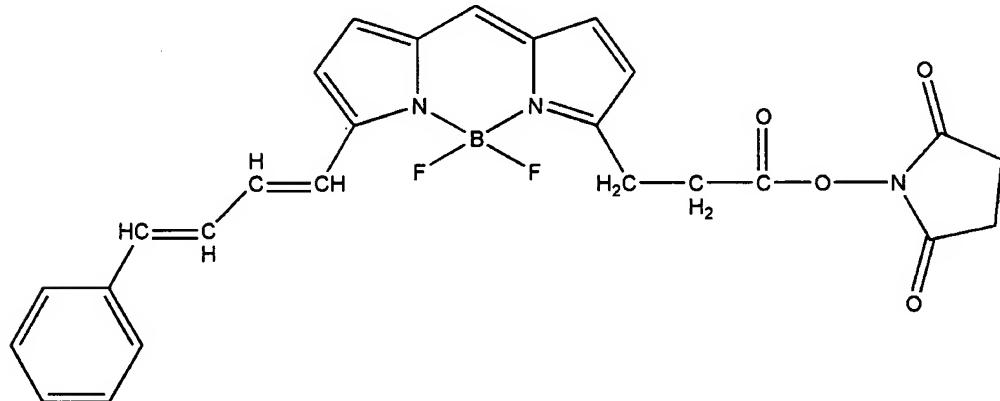


24. A method, as claimed in Claim 20, wherein the dipyrrometheneboron difluoride dye has the formula:



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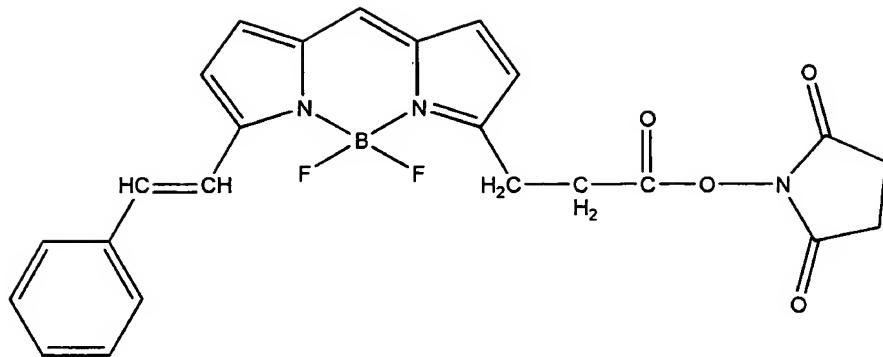
25. A method, as claimed in Claim 20, wherein the dipyrrometheneboron difluoride dye has the formula:



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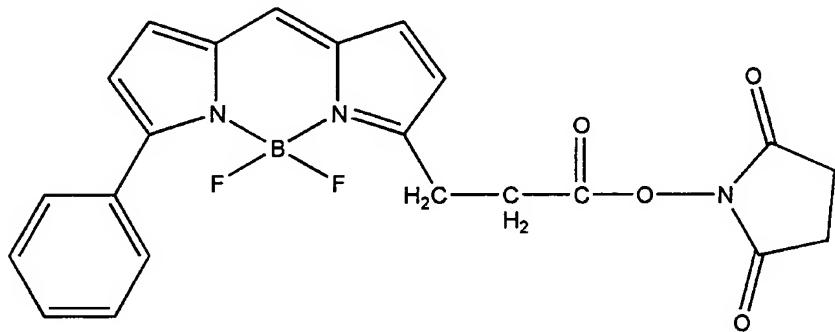
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26. A method, as claimed in Claim 20, wherein the dipyrrometheneboron difluoride dye has the formula:



27. A method, as claimed in Claim 20, wherein the dipyrrrometheneboron difluoride dye has the formula:

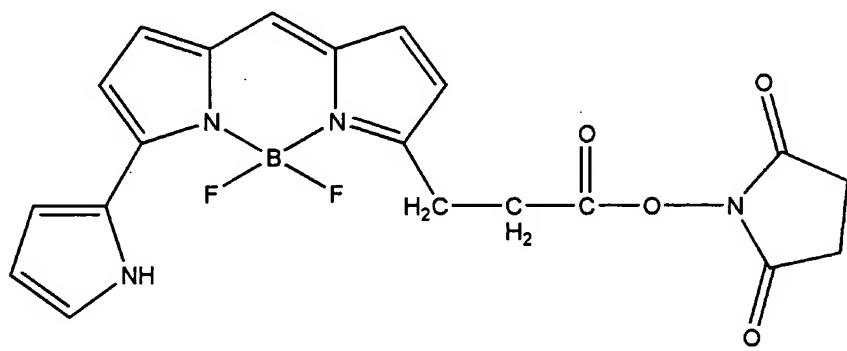
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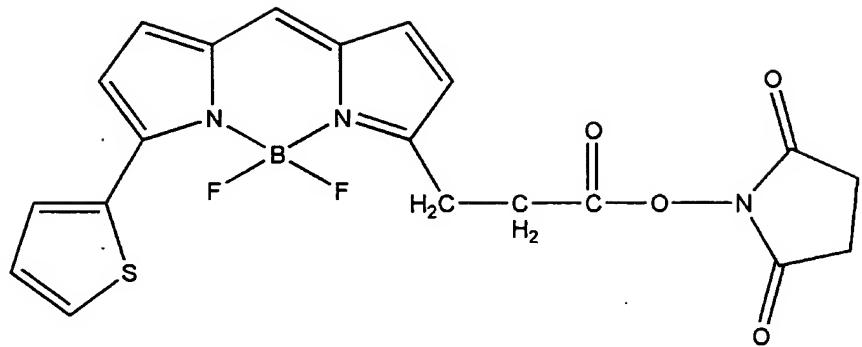
28. A method, as claimed in Claim 20, wherein the dipyrrrometheneboron difluoride dye has the formula:

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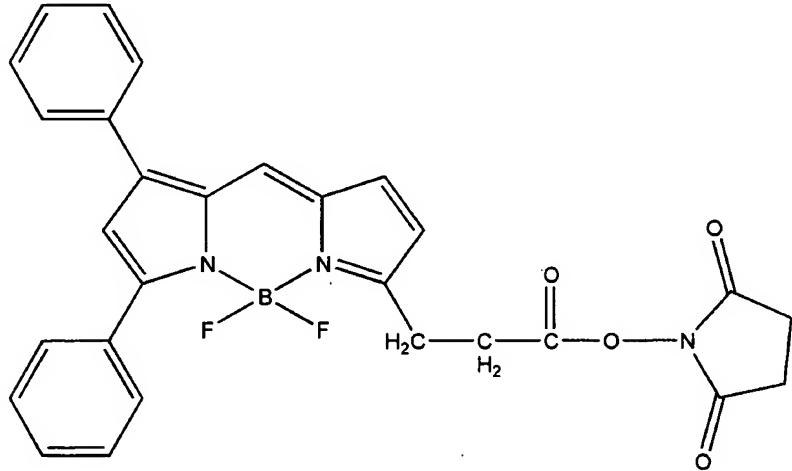
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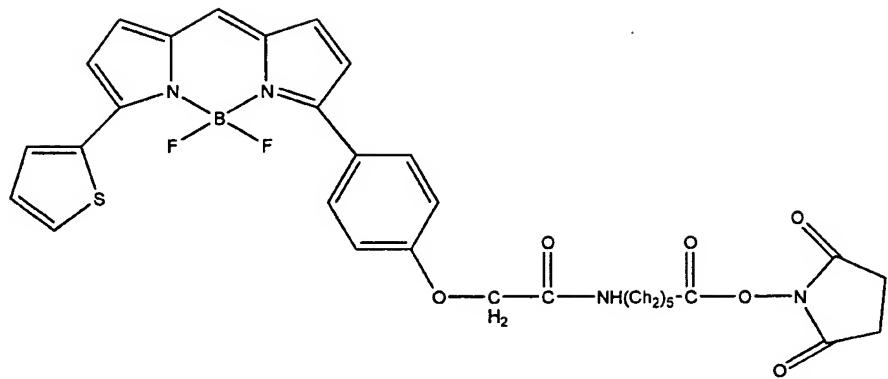
29. A method, as claimed in Claim 20, wherein the dipyrrrometheneboron difluoride dye has the formula:



30. A method, as claimed in Claim 20, wherein the dipyrrrometheneboron difluoride dye has  
5 the formula:



31. A method, as claimed in Claim 20, wherein the dipyrrrometheneboron difluoride dye has  
10 the formula:



32. A method, as claimed in Claim 20, wherein said solid support is made of solvent-resistant materials that are selected from the group consisting of nylon, poly(vinylidene difluoride), glass, plastics, and their derivatives.

5           33. A method, as claimed in Claim 32, wherein said solid support is made of materials that  
are poly(vinylidene difluoride).

10          34. A method, as claimed in Claim 20, wherein said poly(amino acids) on said solid support each have a molecular weight of between 500 Daltons and 200,000 Daltons.

15          35. A method, as claimed in Claim 20, wherein for said dipyrrrometheneboron difluoride dye R<sup>1</sup> is methyl or -L-Rx; R<sup>2</sup> is H, bromine, or -L-Rx; R<sup>3</sup> is H or methyl; R<sup>4</sup> is H or -L-Rx; R<sup>5</sup> is H, methyl, or phenyl; R<sup>6</sup> is H or bromine; and R<sup>7</sup> is methyl, phenyl, alkoxyphenyl, phenylethenyl, phenylbutatdienyl pyrrolyl, or thienyl; where -L- is -(CH<sub>2</sub>)<sub>2</sub>-, -(CH<sub>2</sub>)<sub>4</sub>-, -OCH<sub>2</sub>C(O)NH(CH<sub>2</sub>)<sub>5</sub>-, -(CH<sub>2</sub>)<sub>2</sub>-C(O)NH(CH<sub>2</sub>)<sub>5</sub>-, -(CH)<sub>2</sub>C<sub>6</sub>H<sub>4</sub>OCH<sub>2</sub>C(O)NH(CH<sub>2</sub>)<sub>5</sub>-, and Rx is a succinimidyl ester of a carboxylic acid..

20          36. A method, as claimed in Claim 35, wherein said dipyrrrometheneboron difluoride dye is present in the labeling mixture at a concentration of 0.10 micromolar to 10 micromolar, and

wherein said labeled poly(amino acids) are illuminated for five seconds or less.

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37. A method, as claimed in Claim 20, further comprising adding a specific binding pair member that selectively binds to a target within said immobilized poly(amino acids) that is its complementary binding pair.

5 38. A method, as claimed in Claim 37, where said specific binding pair member contains a label that is an enzyme or a hapten.

39. A method, as claimed in Claim 37, where said specific binding pair member contains a label that is a fluorophore.

10

40. A method, as claimed in Claim 37, further comprising:  
adding a secondary complementary binding pair member that contains a label and that selectively binds to the specific binding pair member.

15

41. A method, as claimed in Claim 40, wherein the label on the secondary complementary binding pair is an enzyme.

42. A method, as claimed in Claim 40, wherein the label on the secondary complementary binding pair is a fluorescent dye.

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43. A method, as claimed in Claim 41, wherein said enzyme is a peroxidase or a phosphatase.

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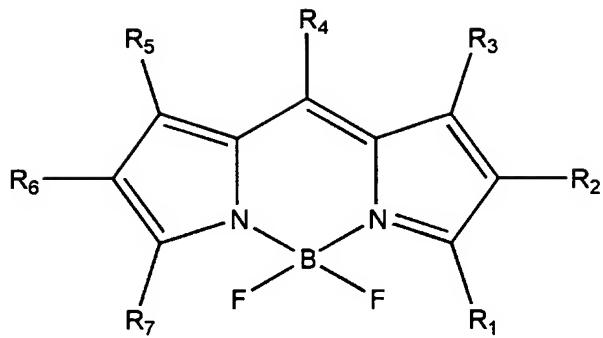
44. A method, as claimed in Claim 43, wherein said peroxidase is horseradish peroxidase.

45. A method, as claimed in Claim 43 wherein said phosphatase is alkaline phosphatase.

30

46. A method, as claimed in Claim 41, wherein said enzyme is capable utilizing a fluorogenic substrate to generate a detectable optical response.

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47. A method, as claimed in Claim 46, wherein said enzyme is a peroxidase and said fluorogenic substrate is a fluorescent tyramide.
48. A method, as claimed in Claim 46, wherein said enzyme is a phosphatase and said fluorogenic substrate is a quinazolinone phosphate.
49. A method, as claimed in Claim 46, wherein said enzyme is a phosphatase and said fluorogenic substrate is 9H-(1,3-dichloro-9,9-dimethylacridin- 2-one-7-yl) phosphate.
- 10 50. A method, as claimed in Claim 46, wherein said enzyme is a peroxidase and said fluorogenic substrate is a polyfluorinated xanthene.
- 15 51. A method, as claimed in Claim 40, wherein said secondary complimentary binding pair is an antibody or an antibody fragment.
52. A method, as claimed in Claim 39, wherein said complementary specific binding pair member is a lectin.
- 20 53. A method, as claimed in Claim 39, wherein said specific binding pair member is biotin-binding protein that contains a label.
54. A method, as claimed in Claim 53, wherein said biotin-binding protein is streptavidin.
- 25 55. A method, as claimed in Claim 53, wherein said biotin-binding protein is NeutrAvidin.
56. A method, as claimed in Claim 37, wherein said specific binding pair member is an antibody or antibody fragment, an aptamer, a lectin, or a biotin-binding protein.
- 30 57. A kit for detection of poly(amino acids) immobilized on a solid surface, said kit comprising:  
a. a dipyrrometheneboron difluoride dye of the formula:



wherein each of R<sup>1</sup> through R<sup>7</sup> are independently selected from the group consisting  
 5 of H, halogen, L-Rx, and substituted or unsubstituted C<sub>1</sub>-C<sub>6</sub> alkyl, aryl, arylethenyl,  
 arylbutadienyl, and heteroaryl;

provided that one or more of R<sup>1</sup> through R<sup>7</sup> is H,

two or more of R<sup>1</sup> through R<sup>7</sup> is nonhydrogen, and

only one of R<sup>1</sup> through R<sup>7</sup> is -L-Rx, where L is a spacer having 1-24 nonhydrogen  
 10 atoms selected from the group consisting of C, N, O, P, and S and is composed of  
 any combination of single, double, triple or aromatic carbon–carbon bonds, carbon–  
 nitrogen bonds, nitrogen–nitrogen bonds, carbon–oxygen bonds, carbon–sulfur  
 bonds, phosphorus–oxygen bonds, and phosphorus–nitrogen bonds; and Rx is a  
 reactive group that is a maleimide or a succinimidyl ester of a carboxylic acid;  
 such that the dipyrrometheneboron difluoride dye has an absorption maximum  
 15 between 495 nm and 640 nm;

b. a specific binding pair member that contains a label and that selectively binds to a target  
 that is its complementary binding pair.

20

58. A kit, as claimed in Claim 57, wherein the specific binding pair member contains a  
 label that is an enzyme; wherein said enzyme is capable utilizing a fluorogenic substrate to  
 generate a detectable optical response, said kit further comprising the fluorogenic substrate.

25

59. A kit, as claimed in Claim 57, wherein said specific binding pair member is an antibody  
 or antibody fragment.

60. A kit, as claimed in Claim 57, wherein the specific binding pair member contains a label that is a fluorescent dye.

5 61. A kit, as claimed in Claim 57, wherein said specific binding pair member is a biotin-binding protein.

62. A kit, as claimed in Claim 61, wherein said biotin-binding protein is avidin, Neutravidin or streptavidin.

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63. A kit, as claimed in Claim 58, wherein said label is an enzyme that is a peroxidase or a phosphatase.

64. A kit, as claimed in Claim 63, wherein said peroxidase is horseradish peroxidase.

15

65. A kit, as claimed in Claim 64, wherein said fluorogenic substrate peroxidase substrate that is a fluorescent tyramide.

66. A kit, as claimed in Claim 63, wherein said phosphatase is alkaline phosphatase.

20

67. A kit, as claimed in Claim 66, wherein said fluorogenic substrate is a phosphatase substrate that is a 9H-(1,3-dichloro-9,9-dimethylacridin-2-one-7-yl) phosphate.

25 68. A kit, as claimed in Claim 66, wherein said fluorogenic substrate is a phosphatase

substrate that is a 2-(5'-chloro- 2'-phosphoryloxyphenyl)-6-chloro- 4(3H)-quinazolinone.

69. A kit, as claimed in Claim 66, wherein said fluorogenic substrate is a phosphatase substrate that is ELF 39 reagent.

30 70. A kit, as claimed in Claim 58, wherein

for the dipyrrometheneboron difluoride dye, R<sup>1</sup> is methyl or -L-Rx; R<sup>2</sup> is H, bromine, or -L-Rx; R<sup>3</sup> is H or methyl; R<sup>4</sup> is H or -L-Rx; R<sup>5</sup> is H, methyl, or phenyl; R<sup>6</sup> is H or bromine; and R<sup>7</sup> is methyl, phenyl, alkoxyphenyl, phenylethenyl, phenylbutadienyl pyrrolyl, or thienyl; where -L- is -(CH<sub>2</sub>)<sub>2</sub>- , -(CH<sub>2</sub>)<sub>4</sub>- , -OCH<sub>2</sub>C(O)NH(CH<sub>2</sub>)<sub>5</sub>- , -(CH<sub>2</sub>)<sub>2</sub>-C(O)NH(CH<sub>2</sub>)<sub>5</sub>- ,

5 -(CH)<sub>2</sub>C<sub>6</sub>H<sub>4</sub>OCH<sub>2</sub>C(O)NH(CH<sub>2</sub>)<sub>5</sub>- ;

and Rx is a succinimidyl ester of a carboxylic acid;

the specific binding pair member is an antibody or a streptavidin that contains a label that is an alkaline phosphatase and the fluorogenic substrate is a 9H-(1,3-dichloro-9,9-

10 dimethylacridin-2-one-7-yl) phosphate, a 2-(5'-chloro- 2'-phosphoryloxyphenyl)-6-chloro-4(3H)-quinazolinone, or ELF 39 reagent.

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